Approaches to Developing Alternative and Predictive Toxicology Based on PBPK/PD and QSAR Modeling

Raymond S.H. Yang,[1,2] Russell S. Thomas,[1,2] Daniel L. Gustafson,[1,2] Julie Campain,[1,2] Stephen A. Benjamin,[1,3] Henk J.M. Verhaar,[4] and Moiz M. Mumtaz[5]

[1]Center for Environmental Toxicology and Technology; [2]Department of Environmental Health; [3]Department of Pathology, Colorado State University, Fort Collins, Colorado; [4]Research Institute of Toxicology, Utrecht University, Utrecht, The Netherlands; [5]Agency for Toxic Substances and Disease Registry, Atlanta, Georgia

Systematic toxicity testing, using conventional toxicology methodologies, of single chemicals and chemical mixtures is highly impractical because of the immense numbers of chemicals and chemical mixtures involved and the limited scientific resources. Therefore, the development of unconventional, efficient, and predictive toxicology methods is imperative. Using carcinogenicity as an end point, we present approaches for developing predictive tools for toxicologic evaluation of chemicals and chemical mixtures relevant to environmental contamination. Central to the approaches presented is the integration of physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) and quantitative structure–activity relationship (QSAR) modeling with focused mechanistically based experimental toxicology. In this development, molecular and cellular biomarkers critical to the carcinogenesis process are evaluated quantitatively between different chemicals and/or chemical mixtures. Examples presented include the integration of PBPK/PD and QSAR modeling with a time-course medium-term liver foci assay, molecular biology and cell proliferation studies, Fourier transform infrared spectroscopic analyses of DNA changes, and cancer modeling to assess and attempt to predict the carcinogenicity of the series of 12 chlorobenzene isomers. Also presented is an ongoing effort to develop and apply a similar approach to chemical mixtures using *in vitro* cell culture (Syrian hamster embryo cell transformation assay and human keratinocytes) methodologies and *in vivo* studies. The promise and pitfalls of these developments are elaborated. When successfully applied, these approaches may greatly reduce animal usage, personnel, resources, and time required to evaluate the carcinogenicity of chemicals and chemical mixtures. — *Environ Health Perspect* 106(Suppl 6): 1385–1393 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-6/1385-1393yang/abstract.html*

Key words: physiologically based pharmacokinetic/pharmacodynamic modeling, PBPK/PD modeling, quantitative structure–activity relationship, QSAR, alternative toxicology, predictive toxicology, chemical mixtures

This paper is a presentation of the ongoing development of concepts and approaches toward predictive and alternative toxicology, particularly for chemical mixtures, from our laboratories, with collaboration from U.S. and European scientific colleagues. As a starting point, one asks why such a development is necessary. To answer this question adequately, certain reality checks must be emphasized:

There Is No Such Thing as a Single Chemical Exposure

This issue has been discussed in detail elsewhere (*1–3*). Briefly, a single chemical often ends up with many metabolites in the body. Furthermore, considering the multiple chemicals to which we are commonly exposed in foods, drinks, medicines, cosmetics, and indoor and outdoor pollutants and the fact that even our own body is a chemical mixture, there is really no such thing as a single chemical exposure in anyone's life.

Conventional Toxicology Studies Cannot Keep Pace with Single Chemicals, Let Alone Chemical Mixtures

The National Cancer Institute's Carcinogenesis Bioassay Program was established in 1962 (*4*). This bioassay program was later transferred to the U.S. National Toxicology Program upon its establishment in 1978. In the bioassay program's combined 36 years of operation, approximately 500 chemicals have been studied for carcinogenicity and other chronic toxicities (*5*). These studies and the related range-finding and dose-setting studies are extremely expensive, they require large numbers of animals, and the study duration is long (*6*). Even though these studies are the gold standards, considering the approximately 70,000 to 600,000 chemicals in commerce (*7–9*), the number of chemicals for which we currently have adequate toxicology information for risk assessment is minuscule. At the present mode and rate of study of these chemicals, it is doubtful that our society will ever have a thorough toxicologic evaluation on the majority of the chemicals that are used now or may be used in the future. Further consideration of the issue of health effects of

This paper is based on a presentation at the Conference on Current Issues on Chemical Mixtures held 11–13 August 1997 in Fort Collins, Colorado. Manuscript received at *EHP* 5 June 1998; accepted 8 September 1998.

The research work and related concept development on chemical mixtures were supported in part by a Superfund Basic Research Program Project grant (P42 ES05949) from the National Institute of Environmental Health Sciences, a grant (F49620-94-1-0304) from the Air Force Office of Scientific Research, and a cooperative agreement (U61/ATU881475) from the Agency for Toxic Substances and Disease Registry. Without such generous support for biomedical research this work could never have been possible.

Address correspondence to R.S.H. Yang, Center for Environmental Toxicology and Technology, Colorado State University, Foothills Campus, Fort Collins, CO 80523-1680. Telephone: (970) 491-5652. Fax: (970) 491-8304. E-mail: ryang@cvmbs.colostate.edu

Abbreviations used: BBDR, biologically based dose response; DEN, diethylnitrosamine; GSH, reduced glortathaione; GSSG, oxidized gultathione: GST-P, glutathione *S*-transferase, placental form; LED, lower 95% confidence limit on an effective dose associated with a 5 or 10% extra risk, noted by subscript of 5 or 10, respectively; MLR, multiple linear regression; PBPK/PD, physiologically based pharmacokinetics/pharmacodynamics; PK, PBPK model parameters; PLS, partial least square; QSAR, quantitative structure–activity relationship; SHE, Syrian hamster embryo; TGF, transforming growth factor; TR, toxicologic response.

chemical mixture exposures (i.e., real-world issues), it is impossible to deal with the problems of combination toxicology of chemical mixtures by adopting the approach of systematic conventional toxicology and carcinogenicity testing (*1,10*).

Predictive and Alternative Toxicology Must Be Developed

During our approximately 15 years of active research in the toxicology of chemical mixtures, we have become resigned to the fact that the conventional animal toxicology testing methods will not work for chemical mixtures, particularly in the evaluation of carcinogenicity (*2,3,6*). We believe that to deal with chemical mixture issues effectively we must fully utilize and integrate computational technology, mathematical and statistical modeling, mechanistically based short-term toxicology studies, and cellular and molecular biology methodologies. Furthermore, an efficient experimental approach or system for chemical mixtures must at least meet the following critical requirements: It must be relatively simple, short-term, and inexpensive; be based on the best science; incorporate mechanisms of toxicity; have broad applicability; and have predictive capability.

The Increasing Application of Computer Technology in Toxicology Is Inevitable

Widespread application of computers in risk assessment, particularly the use of linearized multistage and other models for cancer risk assessment, has been a reality for more than two decades. Since the late 1980s, the advancement of physiologically based pharmacokinetic (PBPK) modeling and the integration of PBPK/pharmacodynamic (PD) modeling into the risk assessment process further enhanced the utilization of computer technology. Thus, computer application in toxicology is already a fact. Looking into the future, it becomes obvious that some type of predictive approach must be developed to handle the huge number of single chemicals in commerce and the nearly infinite number of chemical mixtures in the environment. It is inevitable that computer technology will be heavily involved in any development of predictive toxicology.

Prediction of health effects usually involves some type of mathematical modeling, which may range from the classical compartmental pharmacokinetic modeling to the currently advancing PBPK/PD, biologically based dose response (BBDR), and/or quantitative structure–activity relationship (QSAR) modeling. Some successes, including those from our studies, in prediction of pharmacokinetic fate and toxicity of simple chemical mixtures are already evident in the literature (*11–22*). The toxicologic end points of prediction, for instance, include an interaction threshold (*18,19*) and acute lethality due to hepatic injuries (*20*).

For more complex mixtures the integration of PBPK/PD, BBDR, QSAR modeling, and lumping analysis (a modeling tool developed in the petroleum industry) may allow the development of a predictive tool for the health effects (*23*). In the 1960s the application of lumping analysis rendered it possible to predict gasoline production based on a few lumps (i.e., similar groups of chemicals based on carbon numbers or boiling points) rather than the thousands of component chemicals of the petroleum (*24,25*). Thus, even though relatively little is known about the complex mixture of petroleum, a predictive tool was developed and applied from modeling. If a relatively crude lumping analysis was able to predict some aspects of catalytic cracking of petroleum 30 years ago, why can't we attempt to predict health effects from chemical mixtures through a much more sophisticated modeling technique such as structure-oriented lumping (*26,27*)? Accordingly, our laboratory, in conjunction with scientists from other institutions, is actively pursuing this area of research (*28*).

Long-Term, Low-Level Exposures Are Below the Sensitivity of Present-Day Methods of Experimental Toxicology

Conventional toxicology methods are mainly observational and descriptive. As an example, in carcinogenesis the animals, usually rodents, are dosed with the chemical of interest for 2 years, the surviving animals are sacrificed, and the tissues are examined histopathologically for the presence of tumors. Because the number of animals per group is limited, usually fairly high dose levels must be used to elicit tumorigenic responses. In cancer risk assessment these high-dose experimental results are extrapolated to the very low dose (i.e., environmentally realistic) regions where such conventional experimental protocols would not be able to detect any carcinogenic responses. Even with the newly proposed point of departure approach for cancer risk assessment (*29*), the lower 95% confidence limit on a dose associated with 5 or 10% extra risk (LED_{05} or LED_{10}, respectively) region is still not easily attainable with conventional protocols. Moreover, these dose levels (i.e., effective dose, ED_{05} or ED_{10}) are still orders of magnitude higher than the estimated environmental exposure levels.

Recent advances in molecular biology techniques offer unique opportunities for the development of more efficient and sensitive methods for toxicology. For instance, techniques such as polymerase chain reaction or reverse transcriptase polymerase chain reaction, coupled with mechanistic studies of the cancer process in cell culture systems, may detect early or late genotypic changes related to carcinogenesis at dose levels much below the limits of detection of more conventional toxicology methods. As we propose here, the integration of this type of mechanistic data with PBPK/PD, BBDR, and/or QSAR modeling may formulate an efficient and predictive approach for carcinogenic potentials of chemicals and chemical mixtures.

Because this is a review paper, many experimental details are not provided. Readers are referred to the papers cited for additional information. The following discussion will follow the chronological order of development of these approaches in our laboratories.

Experimental Approaches

This portion of the paper follows the evolution of our thinking in the last few years on how to develop predictive approaches for single chemicals and chemical mixtures. To focus our presentation we chose to limit the discussion to carcinogenesis only. Initially, we concentrated our effort on a medium-term (i.e., 8 weeks) *in vivo* experimental approach and its integration with PBPK/PD modeling. Although we made progress on this front and obtained interesting findings, it was soon obvious that even this shorter term, more efficient *in vivo* system was too resource intensive to be routinely used for chemical mixture work. Therefore, still more efficient systems must be developed. We investigated *in vitro* systems with our specific criteria (i.e., relatively simple, short-term, and inexpensive; based on the best science; understanding mechanisms of toxicity; broad applicability; and predictive capability) in mind. We found that cell culture systems offer unique opportunities, particularly in mechanistic and time-course studies related to carcinogenesis. Thus, as a further development, *in vitro* mechanistic studies using cell culture systems and their

integration with pharmacodynamic modeling became a major emphasis in our laboratories. Our present thinking is that cell culture systems and *in vivo* animal studies may work in concert to bring about much-needed mechanistic and pharmacokinetic information for PBPK/PD modeling. When quantitative information on the selected molecular and cellular end points is available on chemicals with structural correlation, QSAR modeling may then be applied to assess their relationship with carcinogenic potentials. In doing so, the positive QSAR relationship between certain end points of a series of chemicals with their respective carcinogenic potentials may be used to develop a predictive approach for other untested chemicals with structural similarities.

The Medium-Term Liver Foci Bioassay

To simplify the detection of carcinogenicity of chemicals and chemical mixtures, Ito and colleagues developed the medium-term liver foci bioassay (*30,31*). The details of experimental protocol of this bioassay may be found in Ito et al. (*30,31*); the bioassay is discussed briefly below.

The medium-term liver foci bioassay (*30,31*) utilizes the placental form of glutathione *S*-transferase (GST-P) as a marker for rat hepatic preneoplastic and neoplastic lesions (*32,33*). The medium-term hepatocarcinogenesis bioassay of Ito et al. (*30,31*), an 8-week experiment, utilizes F344 rats that are given a single dose of diethylnitrosamine (DEN) to initiate carcinogenesis. After a 2-week period the rats are given repeated exposure to a test compound for a dosing period of 6 weeks. At the end of week 3, rats are subjected to partial hepatectomy to maximize opportunities of promotion via a high rate of cell proliferation. All rats are sacrificed at the end of week 8 for evaluation of development of preneoplastic hepatocellular nodules by staining for expression of GST-P (*30,31*). Extensive testing has demonstrated that the induction of GST-P-positive foci in the medium-term bioassay for liver carcinogens correlates well with the incidence of hepatocellular carcinomas in parallel long-term assays (*30,31*). Two hundred seventy-seven chemicals have been tested in Ito et al.'s (*34*) medium-term liver foci bioassay; it has correctly identified 97% of genotoxic hepatocarcinogens and 84% of nongenotoxic hepatocarcinogens (*34*). Thus, for rapid screening of large numbers of chemicals and for reduction in the required number of animals, this assay is of great advantage.

A Modified Time-Course Medium-Term Liver Foci Bioassay

In the original protocol of Ito et al.'s (*30,31*) medium-term liver foci bioassay, the animals undergo terminal sacrifice at the end of the 8-week experimental period and the data are for only one time point. To obtain sufficient data for constructing a viable PBPK/PD model ("Integration of *In Vivo* Studies and PBPK/PD Modeling"), time-course events must be studied. Therefore, we modified the original Ito et al. (*30,31*) protocol into time-course studies based on anticipated cell proliferation rates during this 8-week experimental period (Figure 1). With this modified protocol we obtained pharmacokinetic data, cell proliferation rates, selected molecular and biochemical information (i.e., c-*jun*, c-*fos*, CYP1A2, reduced glutathione (GSH): oxidized glutahione (GSSG) ratios, porphyrin levels) as well as morphometric data on foci at different stages of the foci development following treatment of several individual chlorobenzene isomers. Detailed descriptions of methodologies involved in the measurement of these parameters are discussed in Thomas et al. (*35,36*) and Thomas (*37*).

Integration of *in Vivo* Studies and PBPK/PD Modeling

The PBPK/PD modeling of Ito et al.'s (*30,31*) medium-term liver foci bioassay presented some unique challenges. Whereas the foundation of this modeling approach was laid by using pentachlorobenzene (*37*) as a model system, the principle is applicable for other chemicals or chemical mixtures. The evolution of the approach for a PBPK/PD model for tumorigenesis in our laboratories followed roughly three stages.

The first stage was the most ambitious stage, in which we formulated a model as shown in Figure 2. Because cell-cycle kinetics was incorporated into this model, our initial effort was devoted to a biologically based mathematical model of the effects of partial hepatectomy on cell cycle kinetics (*38*) and a comparison of quantitative immunohistochemical markers for cell-cycle specific changes in F344 rats (*39*). However, it was soon apparent that there were two pitfalls. First, whereas the proliferating cell nuclear antigen staining appeared to work well for the liver cells undergoing rapid proliferation such as after two-thirds partial hepatectomy (*39*), quantitative morphometric analysis would be prohibitively resource-intensive for liver at resting state, which has a low rate of cell turnover. Second, although a biologically motivated model depicting cell-cycle kinetics

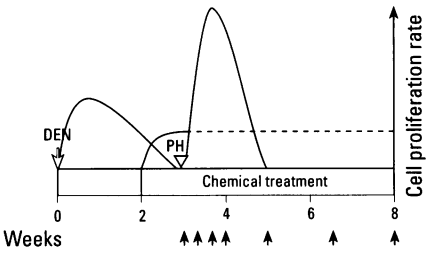

Figure 1. Experimental protocol for the modified time-course of Ito's medium-term liver foci assay (*30,31*). Abbreviations: PH, partial hepatectomy; LI, labeling index. Each arrow under the time axis depicts the following experimental procedures: 1) sacrifice five rats/group/time point following a 3- to 5-day bromodexyuridine exposure; 2) liver tissue for LI measurement; 3) liver tissue for molecular biology studies; 4) tissues for chemical analyses; 5) liver tissue for oxidative stress studies; and 6) histopathology and morphometric analyses on liver.

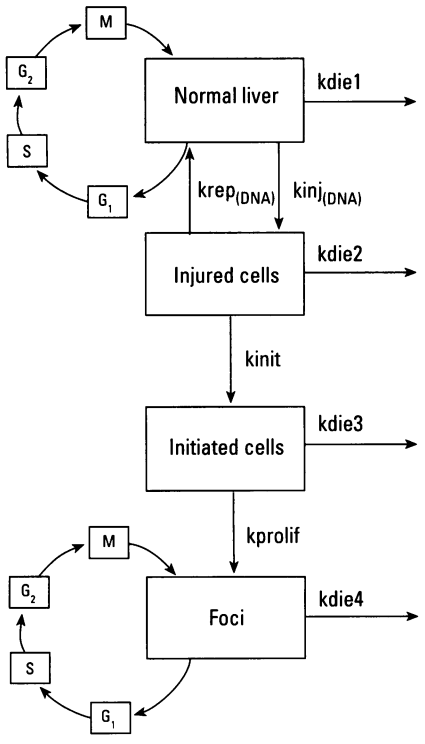

Figure 2. Diagram of a pharmacodynamic model for the liver foci development for selected chlorobenzene isomers. Biologic processes governing cell replication and preneoplastic lesion have been expressed in terms of fundamental cell cycle kinetics within the framework of a multistage model for cancer events. The cellular stages are labeled G_1, S, G_2, and M. $Krep_{(DNA)}$, $kinj_{(DNA)}$, kinit, kprolif are rate constants for DNA repair, DNA injury, cell initiation, and cell proliferation, respectively. Kdie 1 through 4 are rate constants for cell death for the respective compartments.

was constructed and computer simulations were consistent with available literature data (*38*), the data requirements for applying such a model to the specific chemicals and bioassay systems that we were studying were beyond the reach of our available resources and personnel. Thus, we were overly ambitious in our initial modeling attempt.

In the next stage we simplified the model greatly. As shown in Figure 3, this model depicts (from the top to the bottom) that the normal cells have a growth function (P_N) that is related to the total number of cells, their proliferation rate, and time. These cells may die at a death rate (kdie1) or may be injured (DNA damage) by chemicals at the rate of kinj. The cellular repair processes may fix the damage at the rate of krep. The injured cell may go on to become initiated cells (foci) at the rate of kinit or go on to die at the death rate of kdie2. The initiated cells have their growth function (P_I) and death rate (kdie3) as well. This model (Figure 3) was a prototype and we kept it as simple as possible. Using this model, we attempted to simulate the data published by Tatematsu et al. (*40*) on time-course studies of liver foci development and the liver growth following DEN initiation, phenobarbital promotion, and partial hepatectomy. The results are shown in Figures 4 and 5. The model simulations in Figures 4 and 5 (solid lines) are consistent with the experimental data on both the foci development and the liver growth. Although not shown here, the DEN/control (vehicle instead of phenobarbital) showed similar consistent results between computer simulation and experimental data from Tatematsu et al. (*40*).

Our subsequent modeling effort (stage 3) was directed toward the linking of a PBPK model with the first two cellular states of the two-stage cancer model (the Moolgavkar-Venzon-Knudson model). Part one of the modeling effort involved the PBPK simulation of single- and multiple-gavage dose exposures (*37*)—a reflection of the experimental procedures. Part two took into consideration the physiologic changes resulting from two-thirds partial hepatectomy (*37*)—a drastic physiologic state. Part three included linking a deterministic model with a stochastic model and the pharmacodynamic processes involved in the formation of foci (*37*), the initiated cells in the two-stage carcinogenesis model. To date, all these modeling activities were based on using pentachlorobenzene as a model chemical in the modified time-course medium-term liver foci bioassay system. However, the principles derived from this effort may easily be applicable to chemical mixtures.

Our present and future modeling direction aims at going beyond the initiated cell stage—i.e., to look at the processes involved in the later events of the multistage carcinogenesis process. Relevant discussion follows in "The Integration of Mechanistic Information from *in Vitro* and *in Vivo* Studies and Pharmacodynamic Modeling."

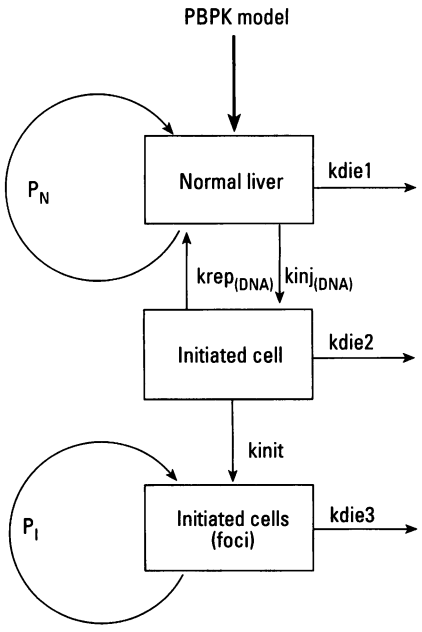

Figure 3. A simplified PBPK/PD model for preneoplastic foci development in the rat liver. See text and Figure 2 for explanation of various functions and rate constants.

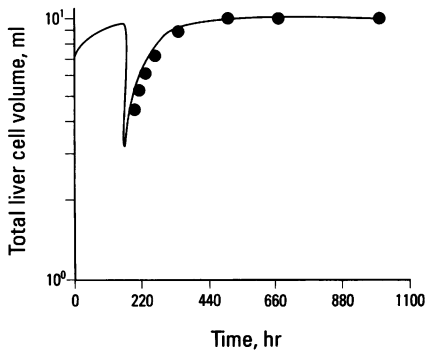

Figure 4. PBPK/PD model simulation of total liver cell volume under the experimental conditions of Ito's liver foci assay using DEN as an initiator and phenobarbital as a promoter. PH was performed on week 3; however, it is shown here at about 168 hr because the simulation (solid line) started at 1 week before PH when phenobarbital treatment began. Data from Tatematsu et al. (*40*).

Studies of Mechanisms of Carcinogenesis Using Cell Cultures following Treatment of Chemicals and/or Chemical Mixtures

Cell culture systems, because of their simplicity and low cost, make it possible to determine the oncogenic potential and mechanisms of transformation of a variety of chemicals and chemical mixtures at the cellular level. Syrian hamster embryo (SHE) cells have the advantage that the carcinogenic potential of many chemicals has been evaluated using morphologic transformation as an index. This has largely been through the effort of scientists at The Procter & Gamble Company (*41,42*). Epidermal keratinocytes are one of the most well-characterized cell culture systems for studying transformation.

For both SHE cells and human keratinocytes, cytotoxicity and altered cellular growth characteristics and induced malignant transformation (i.e., tumorigenicity) are end points for the evaluation of chemical mixtures of interest. In addition, in keratinocytes these studies may be extended to link observed phenotypic changes with alterations in expression of defined cell cycle regulators and growth factors. These biologic experiments should be carried out in an iterative manner with the development of a biologically based model. The concept of model-directed experimentation should be followed to save resources by avoiding unnecessary experimentation.

The overall idea of studying the carcinogenesis process using human keratinocytes

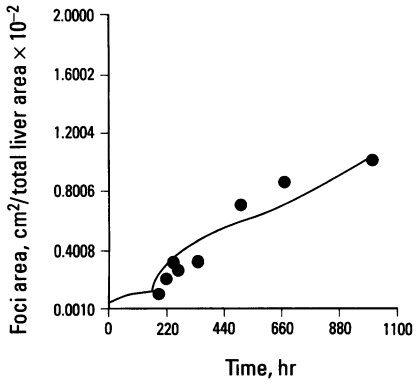

Figure 5. PBPK/PD model simulation of the development of liver foci (foci area) under the experimental conditions of Ito's liver foci assay using DEN as an initiator and phenobarbital as a promoter. PH was performed on week 3; however, it is shown here at about 168 hr because the simulation (solid line) started at 1 week before PH when phenobarbital treatment began. Data from Tatematsu et al. (*40*).

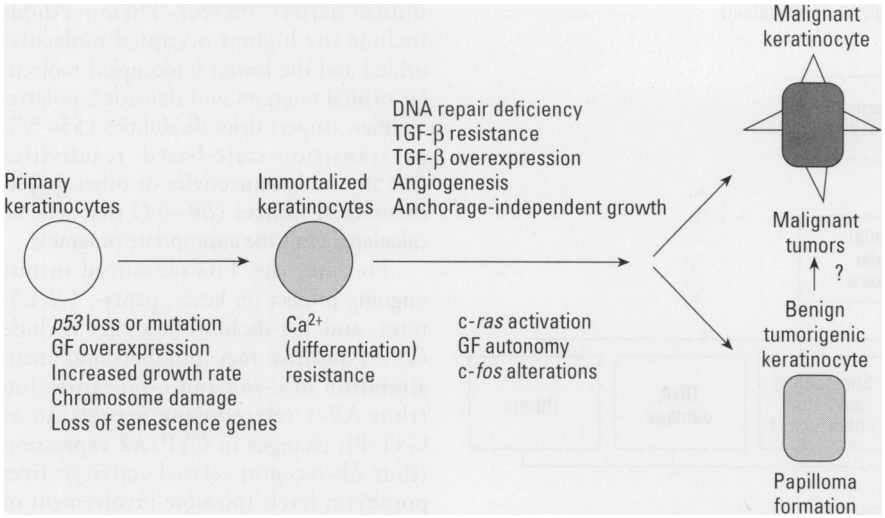

Figure 6. A schematic review of the literature showing phenotypic and genotypic alterations in human keratinocytes during the multistage process of carcinogenesis. GF, growth factor.

is summarized in Figure 6. There are many phenotypic and genotypic changes accompanying the transformation of human keratinocytes; some are early events and others are late events. To cover these changes, commercially available primary human keratinocytes may be used to study the early events (*43*). A number of immortal keratinocyte cell lines such as HaCat (*44*), RHEK-1 (*45*), and NM-1 (*46*) can be used to study the later events. Selected pertinent biomarkers such as transforming growth factor (TGF)-α, TGF-β, c-*myc*, c-*ras*, and *p53* may be studied and quantitative information obtained. Such quantitative information may be utilized to calibrate and verify BBDR models.

Integration of Mechanistic Information from *in Vitro* and *in Vivo* Studies and Pharmacodynamic Modeling

We summarize our approach on the integration of mechanistic information and modeling for the assessment of carcinogenic potential for chemicals in the algorithm in Figure 7.

We believe that because cancer is a cellular process, the carcinogenic potential of chemicals can be identified through the studies of selected key molecular/cellular markers of the cancer processes; also, QSAR and pharmacodynamic modeling of these key biomarkers for cellular process can be effectively utilized to develop a predictive tool for evaluating carcinogenic potential within certain classes of chemicals.

We listed multiple chemicals for chemical mixtures, but this general approach (Figure 7) is applicable for single chemicals as well. The short-term alternative assays may be represented by the SHE cell transformation assay and the modified time-course medium-term liver foci assay (Figure 1); other such tests are available. The molecular or cellular biomarkers from a number of processes leading toward cancer (including loss of growth control, failure to commit apoptosis, loss of DNA repair) may be specifically targeted by a compound or a class of compounds. From time-course studies, critical rate constants (e.g., cellular birth, death, and mutation rates for primary and immortal cells, rates of changes of growth factors, and/or expression of oncogene and tumor-suppressor genes) may be generated for constructing the cancer model(s). Quantitative data on the selected molecular/cellular biomarkers for the cancer process may be used in QSAR modeling for a class of chemicals or chemicals with structural similarities. Such integration of QSAR, PBPK modeling, and cancer modeling (i.e., PBPD modeling) will then provide us with a tool for developing predictive toxicology and risk assessment.

Integration of PBPK/PD and QSAR Modeling for the Development of Predictive Capability

For the last few years, we have devoted a great deal of effort toward developing a predictive toxicology approach using the homolog series of 12 chlorobenzenes. We will use chlorobenzenes as a case study; the same general principles should be applicable to other classes of chemicals.

The chlorobenzenes are a small group of congeneric chemicals. There are 12 congeners in total—from mono- to hexachlorobenzene. Most of these are solid at ambient temperature and pressure, except for mono- and some dichlorobenzenes. Their water solubility is generally low to extremely low, and their hydrophobicity ranges from intermediate to fairly high. The log octanol/water partition coefficient for monochlorobenzene is 2.89, whereas for hexachlorobenzene it is 5.73 (*47*). In a QSAR model, a correlation is developed between one or more quantitative structural, physicochemical, and/or biologic descriptors and a toxicologic end point (*48*). The toxicologic end point can either be quantitative (e.g., an effect level such as the median lethal concentration) or qualitative (e.g., mutagenic or nonmutagenic). Hansch et al. (*49*) provide an interesting overview of the use of QSAR in toxicology.

Our plan is to build and verify QSAR models based on our ongoing studies of molecular and cellular biomarkers of preneoplastic foci formation from exposures to hexa-, penta-, 1,2,4,5-tetra-, and 1,4-dichlorobenzenes; to use these verified QSAR models to predict each of the toxicologic responses and PBPK modeling parameters studied to date for the remaining eight chlorobenzene isomers (1,2,3,4-tetra-, 1,2,3,5-tetra-, 1,2,3,-tri-, 1,2,4,-tri-, 1,3,5-tri-, 1,2-di-, 1,3-di-, and monochlorobenzenes); and to verify the QSAR model predictions by conducting molecular biology/biochemical experiments and PBPK/PD modeling studies on selected chlorobenzene isomers using *in vitro* and *in vivo* methods. To achieve that we will proceed as follows:

The array of toxicologic data on the first four selected chlorobenzenes will be studied using pattern recognition methods (*50,51*) to determine which data correlate with the carcinogenic nature of the compounds. The methods that will be used are principal component analysis, linear discriminant analysis, cluster analysis, and soft independent modeling of class analogy (*50,51*). All of these methods can be used to study which biologic descriptors (or combination of descriptors) are correlated with or even responsible for the carcinogenic nature of the active chlorobenzenes. In the next step the toxicologic end points that are perceived important for the understanding of the carcinogenicity of these compounds will be studied with quantitative methods.

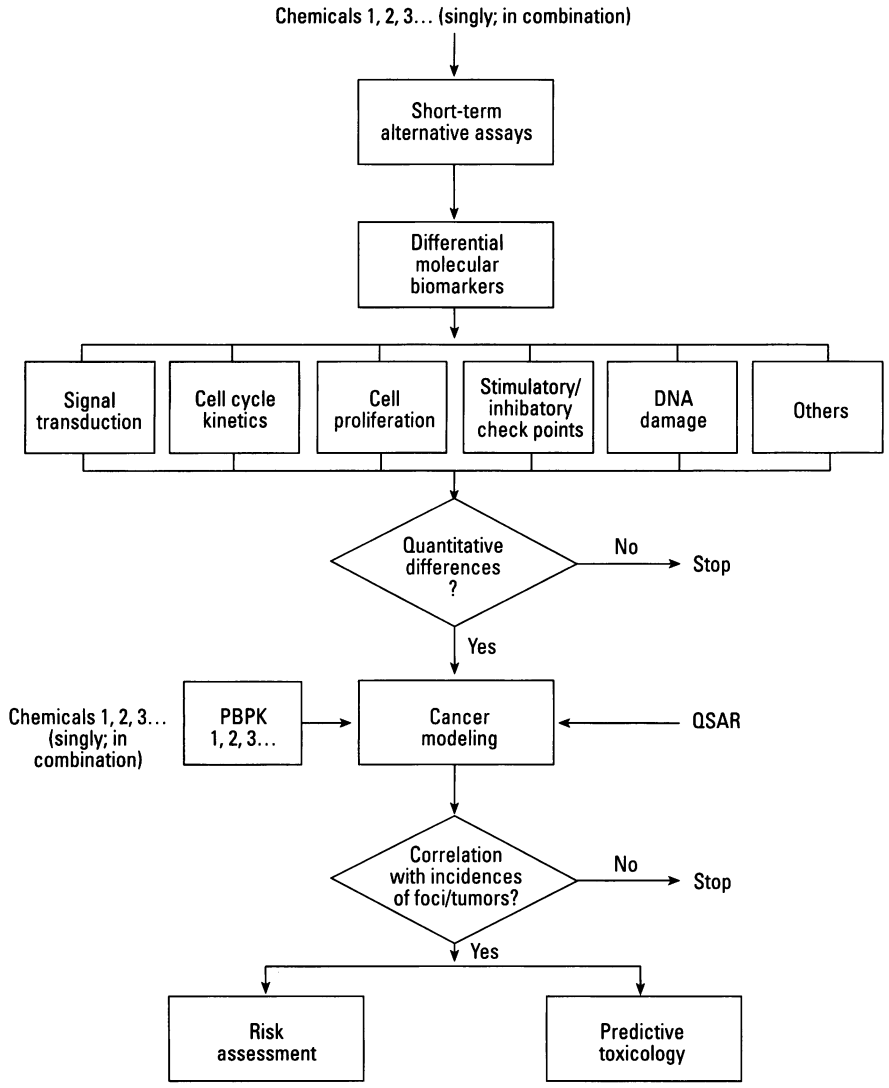

Figure 7. An algorithm for our approach to predictive and alternative toxicology for assessing carcinogenic potentials of chemicals or chemical mixtures.

QSAR correlation and prediction of toxicologic responses on a number of molecular or cellular biomarkers for carcinogenic potentials of the 12 isomers of chlorobenzenes will be carried out in line with the classical Hansch multiple linear regression (MLR) analysis approach (*52–54*). The correlation developed will be similar to the following description, with the understanding that actual relationships will be calculated using partial least square (PLS) regression analysis (a projection multivariate technique) rather than MLR.

$$\log\frac{1}{TR} = a\alpha + b\beta + c\gamma + d\sigma + \ldots$$

This equation, in a general sense, expresses that the toxicologic response (*TR*) measured is a function of a number of factors ($a\alpha$, $b\beta$, $c\gamma$, $d\delta$, ...). Here, a, b, c, d, ... are QSAR model coefficients that may be derived after the model verification from data obtained with the four isomers (i.e., hexa-, penta-, 1,2,4,5-tetra- and 1,4-dichlorobenzenes) that we are currently studying, whereas α, β, γ, δ, ... are the specific numeric derivations of physicochemical parameters retrieved from literature and/or comprehensive databases. Examples of α, β, γ, δ, ... are log K_{ow} (hydrophobicity), melting point, vapor pressure, or Swain–Scott parameters. The latter are electronic parameters generated by theoretical chemical methods–quantum chemical calculations and several reactivity/interaction indices derived thereof. These methods include the highest occupied molecular orbital and the lowest unoccupied molecular orbital energies and densities, polarizabilities, (super) delocalizabilities (*55–57*), or transition-state-based reactivities (*58,59*), and connectivity or other graph-theoretical indices (*60–64*) that will be calculated with the appropriate programs.

To date, the TRs measured in our ongoing project on hexa-, penta-, 1,2,4,5-tetra- and 1,4-dichlorobenzenes include GST-P-positive foci (numbers and area); alteration of c-*jun* and c-*fos* expression (thus AP-1 related gene expression of GST-P); changes in CYP1A2 expression (thus Ah-receptor related activity); liver porphyrin levels (possible involvement of oxidative stress); alterations in the GSH:GSSG ratio; and induction of DNA damage (via Fourier transform infrared analyses). Furthermore, time-course changes were also obtained for liver cell proliferation rates under the influence of DEN, chlorobenzene treatment, and partial hepatectomy in the modified time-course medium-term liver foci bioassay (Figure 1). All these TRs are either quantitative or semiquantitative parameters; thus, they are amenable to QSAR analysis. Of course, judgment on the potency and importance of these TRs must be made with respect to the toxicologic end point, in this case the foci formation.

For QSAR correlation and prediction of PBPK modeling parameters on the 12 isomers of chlorobenzenes, we also intended to apply the following general QSAR model for all relevant PBPK parameters:

$$\log\frac{1}{PK} = a\alpha + b\beta + c\gamma + d\sigma + \ldots$$

where *PK* represents PBPK model parameters measured or estimated.

The PKs measured or estimated to date in our ongoing project on hexa-, penta-, 1,2,4,5-tetra- and 1,4-dichlorobenzenes include tissue partition coefficients for liver, fat, muscle (representing a slowly perfused tissue group), and kidney (representing a rapidly perfused tissue group) and *in vivo* metabolic rate constants (i.e., K_m and V_{max}). The priority of selecting PKs for QSAR modeling will be based on sensitivity analyses in PBPK modeling as to their respective influences on computer simulations; the greater the influence, the higher the priority.

The work of de Jongh and co-workers (*65*) showed that for a large set of diverse chemicals, in both rats and humans, the tissue:blood partition coefficients could be described by a general function of the compound's log K_{ow} of the following form:

$$P_{t,b} = \frac{f_{w,t} + f_{l,t} \times (K_{ow})^a}{f_{w,b} + f_{l,b} \times (K_{ow})^b} + C$$

where $P_{t,b}$ is the tissue to blood partition coefficient, $f_{w,t}$ is the fraction of water in the tissue, $f_{l,t}$ is the fraction of lipid in the tissue, $f_{w,b}$ is the fraction of water in the blood, $f_{l,b}$ is the fraction of lipid in the blood, a and b are exponents, and C is an adjustment factor to provide for the effect of protein binding. This methodology will be used for QSAR modeling of tissue partition coefficients. For metabolic rate constants, the Hansch-like approach using PLS as described earlier.

Conclusion

In this paper we described the approaches for the possible development of a predictive tool for the carcinogenic potential of chemicals and chemical mixtures. For illustrative purposes we used single chemicals (i.e., chlorobenzenes) to develop the concept and approach for predicting carcinogenic potentials of different isomers of chlorobenzenes. Toxicologic interactions may be at the levels of pharmacokinetics and/or pharmacodynamics. Those toxicologic interactions that are relevant to the end point (in this case carcinogenicity) may be linked for different chemicals in a chemical mixture via PBPK/PD modeling. QSAR modeling may be used to extrapolate to other chemicals that are structurally similar to the components of a chemical mixture under study. In this integrated manner we may develop predictive capabilities for the toxicology of chemical mixtures.

How do we approach the issue of risk assessment once such modeling and the generation of quantitative information are realized? The linchpin of a realistic risk assessment is an accurate estimate of target tissue dosimetry from which a virtually safe or safe exposure dose may be calculated. PBPK/PD or BBDR models are powerful tools to define such target tissue dosimetry. For chemical mixtures, three scenarios can be considered. In the first case where a simple chemical mixture with known mechanism(s) of toxicologic interaction, PBPK/PD or BBDR modeling of the toxicologic interaction can provide estimates on an interaction threshold, which may in turn be used as a benchmark dose (e.g., LED_{01} or LED_{05} for a chemical mixture) for risk assessment. In the second case, a chemical mixture (most likely a more complex one with structurally similar components such as a hydrocarbon mixture) may provide quantitative changes of many biomarkers for a toxicologic process as well as defined toxicity for a specific end point. Although the mechanism(s) of interaction may not be entirely clear, PBPK/PD or BBDR modeling may be utilized to estimate the target tissue dosimetry of this chemical mixture as a lump (i.e., a pseudosingle chemical) or as an average chemical based on the average characteristics of such a mixture. In the third case a chemical mixture may have completely different composition including metals, aliphatics, and aromatics. The principles and approaches stated previously may still apply as long as the like components are lumped and quantitative toxicologic information can be obtained for each of the lumps.

The role of QSAR modeling in the overall scheme is to provide interpolations and extrapolations for estimated values for TRs and PKs for those chemicals that are not studied experimentally in a class of chemicals (such as chlorobenzenes) or chemical mixtures (such as hydrocarbons, metals, etc.). These gaps may be filled using QSAR based on structural differences and the related physicochemical changes. In doing so we may carry out PBPK/PD or BBDR modeling on these chemicals or chemical mixtures on an a priori basis.

Discussion in this paper consists of past and ongoing studies as well as theoretical deliberations. Much of the development is still at an embryonic stage. We invite criticisms and suggestions for further modification and refinement of our approaches. Through this type of process we will be able to work toward the goal of establishing a scientifically credible predictive and alternative toxicology.

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